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The Role of B-cell Receptor Signaling in Lymphoid Malignancies

Význam signalizace B-buněčného receptoru u lymfoidních malignit

Bachelor´s thesis

Supervisor
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Prohlášení

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Poděkování

Ráda bych poděkovala především svému školiteli MUDr. Ondřeji Havránkovi Ph.D. za cenné rady, vstřícnost a přátelský přístup při vypracování této práce. Velké dík patří také mé rodině, partnerovi a přátelům za jejich podporu a pozitivní myšlenky v průběhu celého studia.

Abstrakt

Cílem této práce je shrnutí dosavadních poznatků nádorově specifické signalizace B-buněčného receptoru (BCR) a souvisejících možností léčby B-buněčných malignit. Tato signalizace je popsána zejména u non-Hodgkinských lymfomů (NHL). Patogenní signalizace z BCR je podobná signalizaci u normálních B lymfocytů, a to jak antigenem spouštěné, tak i tzv. tonické. Rozdíly mezi antigenně-závislou a antigenně nezávislou BCR signalizací jsou velmi dobře patrné zejména u podtypů difúzního velkobuněčného B lymfomu, který patří mezi nejčastěji diagnostikované NHL. Kromě klasických způsobů léčby jsou pro terapii lymfoidních malignit schválené BCR inhibitory cílené na BTK a PI3K, avšak některé s vysokým stupněm toxicity. Přesto jsou BCR inhibitory pro svůj potenciál v léčbě a vysokou specifičnost hlavním cílem současného výzkumu. Pro zlepšení výsledků léčby NHL je potřeba lepšího porozumění deregulace BCR signalizace a celkové patogeneze nádoru.

Klíčová slova: B-buněčné malignity, non-Hodgkinovy lymfomy, B-buněčný receptor, nádorová signalizace, cílená léčba, inhibitory

Abstract

The aim of this thesis is to review current knowledge about tumor-specific B cell receptor (BCR) signaling and related novel therapy options in B-cell malignancies with the main focus on non-Hodgkin lymphomas (NHL). To a certain degree, the pathogenic BCR signaling mirrors normal forms of BCR signaling, antigen-induced and tonic. Differences between antigen-dependent and antigen-independent forms of BCR signaling are well characterized in two major subtypes of diffuse large B-cell lymphoma, the most common type of NHL. In addition to the conventional chemotherapy, several BCR inhibitors targeting BTK and PI3K have been approved for the treatment of lymphoid malignancies. However, improvements in the tumor specificity, toxicity profiles and patients selection are needed. A better understanding of BCR signaling deregulation and overall tumor pathogenesis is believed to further improve NHL treatment outcomes.

Keywords: B-cell malignancies, non-Hodgkin lymphoma, B-cell receptor, tumor signaling, targeted therapy, inhibitors

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List of abbreviations

ABC DLBCL – activated B-cell-like subtype of DLBCL

AKT – protein kinase B (also known as PKB)

BCAP – B-cell adapter for PI3K

BCR – B-cell receptor

BL – Burkitt lymphoma

BLNK – B-cell linker protein

BTK – Burton tyrosine kinase

C domain – a constant domain of an immunoglobulin

CBM – complex of proteins CARD11-BCL10-MALT1

CDR – complementarity determining region

CLL – chronic lymphocytic leukemia

DAG – diacylglycerol

DLBCL – diffuse large B-cell lymphoma

EBV – Epstein Barr Virus

FDA – U.S. Food and Drug Administration

FL – follicular lymphoma

FOXO1 – forkhead box protein O1

FR – framework (relatively constant sequence)

GCB DLBCL – germinal center B-cell-like subtype of DLBCL

GEP – gene expression profiling

Ig – immunoglobulin

IgH – immunoglobulin heavy chain

IgL – immunoglobulin light chain

ITAM – immunoreceptor tyrosine-based activation motif

ITIM – immunoreceptor tyrosine-based inhibition motif

IL-1 – interleukin-1

IRAK – family of interleukin-1-associated kinases

IKK – IκB kinase

IP3 – inositol triphosphate

MGZL – marginal zone lymphoma

mTOR – mammalian target of rapamycin

mTORC1 – mammalian target of rapamycin complex 1

NF-kB – nuclear factor-kappa B

NFAT – nuclear factor of activated T-cells

NHL – non-Hodgkin lymphoma

PI3K – phosphatidylinositol 3-kinase

PIP3 – phosphatidylinositol (3,4,5)-triphosphate

PKC – protein-tyrosine kinase C

PLC – phosphoinositide phospholipase C

PTEN – phosphatase and tensin homolog

PTK – protein-tyrosine kinase

SFKs – Src-family kinases

SH2 – Src-Homology 2

SYK – spleen tyrosine kinase

SLL – small lymphocytic lymphoma

TIR domain – Toll / IL-1 receptor domain

TLR – toll-like receptor

TRAF6 – TNF receptor-associated factor

TNFAIP3 – TNF alpha-induced protein 3

V domain – a variable domain of an immunoglobulin

WHO – World health organization

1 Introduction

The B cell receptor (BCR) plays an essential role in the development, maturation, and survival of normal B cells. Mature B-cells express BCRs on the plasma membrane, where they recognize and bind antigens with consequent further maturation of B cells into antibody-producing plasma cells and memory B cells. Recently, dysregulation of BCR signaling has been shown to be a driving force in the pathogenesis of many B-cell malignancies, however, the principle of signal initiation in individual types of lymphoid malignancies is not entirely known. In this thesis, I will focus on the most frequent types of lymphomas/leukemias derived from B lymphocytes, where the oncogenic function of BCR was characterized. It is a group of non-Hodgkin lymphomas (NHL) and chronic lymphocytic leukemia (CLL).

The type of BCR signaling in NHL (and CLL) varies according to its dependence on antigen-binding and can be divided into antigen-dependent (chronic active) and antigen-independent (tonic) BCR signaling. In the thesis, I describe the main differences between these two types of BCR signaling and highlight the importance of a detailed understanding of their mechanism for better treatment outcomes in the future.

The importance of BCR signaling is supported by the clinical efficacy of pharmacological inhibition of individual members of the BCR signaling and related pathways, especially PI3K/AKT and BTK. The advantage of BCR inhibitors is their high specificity due to the targeted inhibition of B cell-specific signaling pathways leading to fewer side effects. Several BCR inhibitors are already used in clinical practice and many more are in clinical trials. The efficacy of individual inhibitors in NHLs (and CLL) varies and depends on the type of BCR signaling.

2 Structure and function of BCR

BCR complex consists of a membrane-bound immunoglobulin (Ig) and two associated co-receptor membrane proteins, the CD79α (Igα) and CD79β (Igβ). The immunoglobulin molecule is composed of two heavy (IgH) and two light (IgL) chains linked together by a disulfide bridge. The IgH consists of four domains and IgL of two domains. At the N-termini of both IgH and IgL chains are localized variable (V) regions, forming together the antigen-binding site. The other, non-variable, domains are called constant (C) domains. IgH C determines the class of an Ig (IgA, IgG, IgD, IgE, and IgM) according to which IgH constant domain coding gene is used: α, γ, δ, ε and μ. Cytoplasmic parts of CD79α and CD79β proteins contain specific amino acid sequences critical for signal transduction called Immunoreceptor Tyrosine-based Activation Motif (ITAM).[1]

The BCR has two major functions that are both essential for the functioning of a B-cell: The first is signal transduction and initiation of signaling cascades, which is explained in more detail below. The second is the processing of a target antigen and the presentation to T-helper cells by MHC II molecules. A correctly expressed BCR at the surface of B cell is necessary for its survival even without antigen stimulation and its deletion leads to apoptosis.[2]

3 BCR signaling in normal B cells

There are two basic types of BCR signaling and multiple signaling cascades activated by the BCR. The two major types of BCR signaling are active, triggered by antigen binding, and tonic, providing basic survival signals.

Upon binding of an antigen to the BCR, the receptor transmits a signal via CD79α and CD79β to the intracellular space of the B cell, leading to the activation of protein-tyrosine kinases (PTKs).[3] PTKs are stimulated not only in BCR signaling but also in other cellular mechanisms affecting basic cellular processes. For the physiological functionality of the cell, PTKs require tight regulation of their activity. Constitutive activation of PTK can lead to tumorigenesis. An important PTK family in BCR signaling is the Src-family of tyrosine kinases (SFKs), including LYN kinase. SFKs are localized in lipid rafts, structural and functional subdomains of plasma membranes. Cross-linking of the BCR leads to its relocation to the sites of lipid rafts, where the LYN kinase initiates the signaling cascade and together with other SFKs (FYN and BLK) phosphorylates the tyrosine residues in CD79α/β ITAMs. In addition, SFKs

engagement lead to the activation of CD19, mostly associated in a complex with CD21 (CD19/CD21 complex).[4,5] (FIGURE 1)

LYN kinase is also involved in the inhibition of BCR signaling by phosphorylation of Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) present in the cytoplasmatic parts of inhibitory receptors, e.g., CD22.[6]

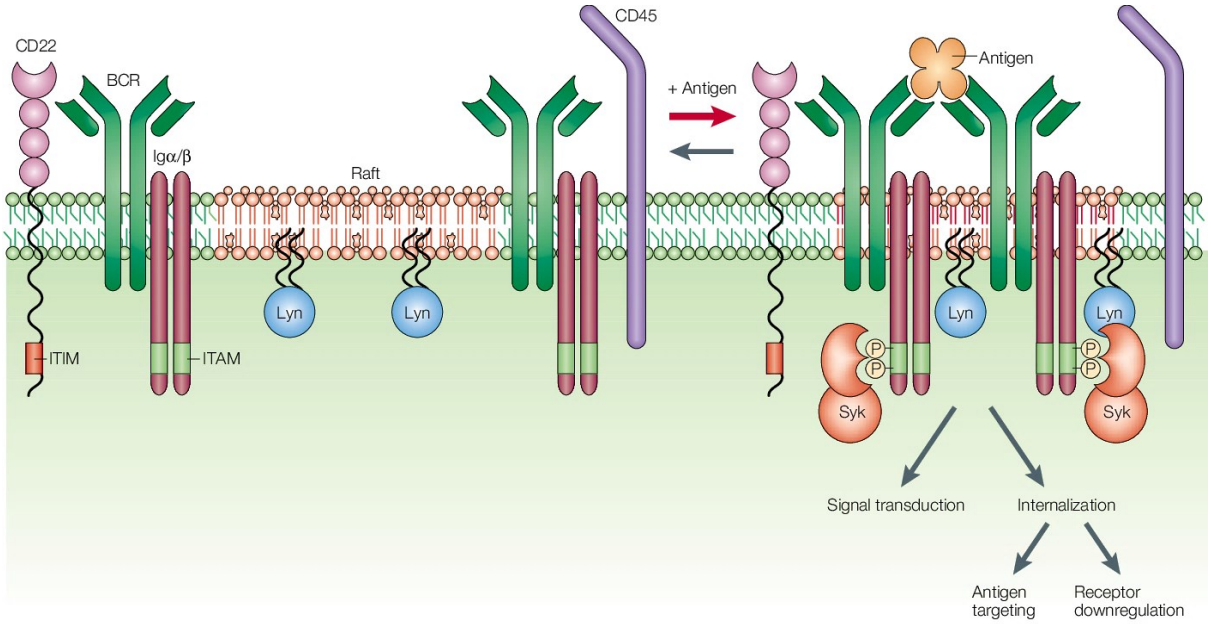

FIGURE 1. Model of BCR relocation to the sites of lipid rafts upon crosslinking, followed by the initiation of the BCR signaling by SFKs (LYN).
Source: Pierce 2002,[94] with permission from Springer Nature.

In contrast to the BCR crosslinking theory, an alternative theory describes BCR as stable molecules with the ability to assemble oligomers, the so-called BCR oligomer complex. Antigen binding alters the conformation of the tight complex, spatially allowing the access to the CD79α/β ITAMs and their consequent phosphorylation.[7]

Both ITAMs need to be symmetrically phosphorylated to be capable of binding the Src-Homology 2 (SH2) domains of the spleen tyrosine kinase (SYK).[8] SYK binding via its SH2 domain is followed by its activation and engagement of B-cell linker protein (BLNK), VAV, and B-cell adapter for PI3K (BCAP).[9] Activated BCAP and/or CD19 are able to recruit phosphatidylinositol 3 kinase (PI3K), one of the major signaling BCR pathways. PI3Ks are divided into three classes depending on their structure and functional properties. The most important in BCR signaling is the first-class PI3K, which consists of a catalytic subunit p110 and

regulatory subunit p85. The consequent production of the phosphatidylinositol triphosphate (PIP3) leads to the activation of Burton's tyrosine kinase (BTK) and protein kinase B (PKB, also known as AKT) and its downstream target mammalian target of rapamycin (mTOR).[10,11,12] (FIGURE 2)

Phosphatase and tensin homolog (PTEN) is the most important regulatory member of the PI3K/AKT pathway. Its catalytic function promotes dephosphorylation of PIP3, therefore, negatively regulating the PI3K/AKT pathway. PTEN dysfunction is frequently involved in the tumorigenesis of B-lymphoid malignancies.[13]

BTK and SYK are involved in the activation of another pathway activated from BCR, Phosphoinositide phospholipase C (PLC) γ2. Second messengers, DAG and IP3, formed at the beginning of the PLC-γ2 pathway, play a major role in the stimulation of intracellular Ca^{2+} mobilization and the activation of protein tyrosine kinases (PKCs), leading to the recruitment of nuclear factor-kappa B (NF-κB) and nuclear factor of activated T-cells (NFAT) transcription factors.[14,15,16] (FIGURE 2)

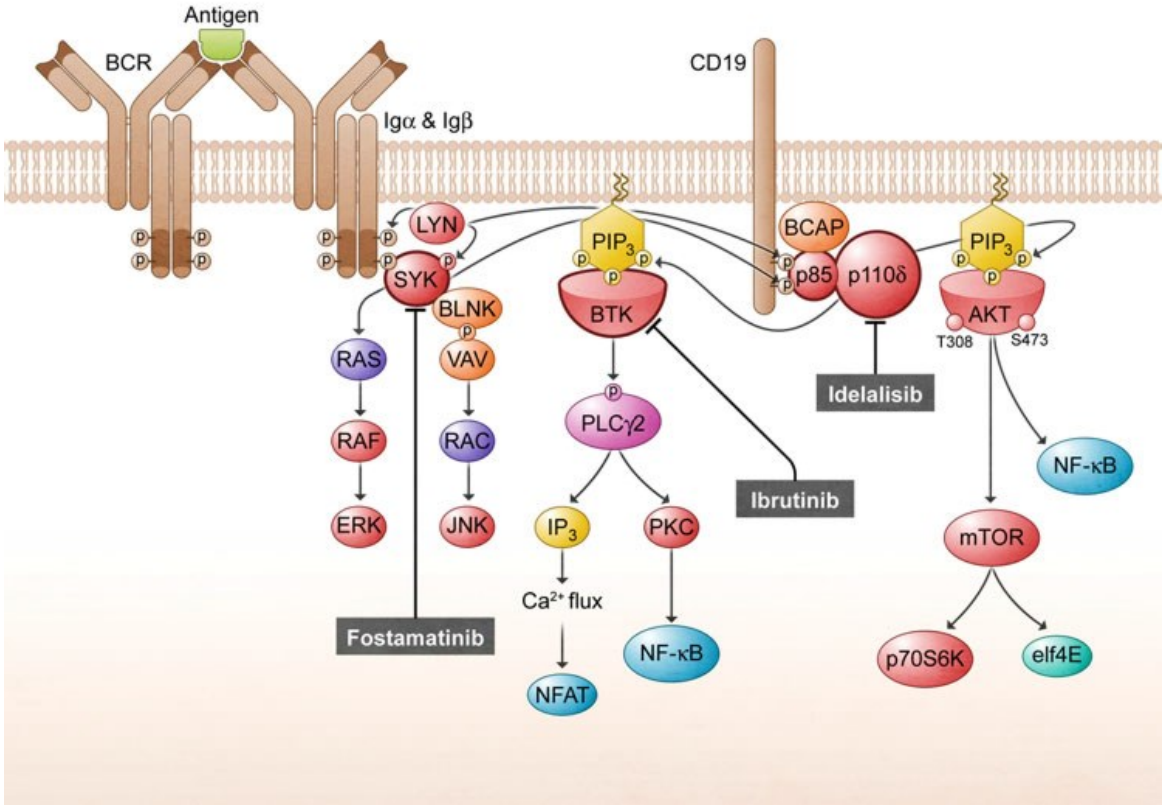

FIGURE 2. Model of BCR signalling with examples of clinically used BCR signaling inhibitors. Source: Puri et al. 2013,[95] with permission from Taylor & Francis.

The BCR transmits signals also in the absence of an antigen. This type of signaling is called a tonic BCR signaling, which is essential for the survival of all mature B-cells. The tonic BCR signal is transmitted also via CD79α/β resulting in CD19 mediated activation of the PI3K signaling pathway.[17] This type of signaling is described in detail below.

4 Lymphoid malignancies

Malignancies of lymphoid cells are a group of heterogeneous diseases that arise from the accumulation of gene mutations and structural or numerical chromosomal aberrations affecting lymphoid cells at different stages of differentiation. They show differences not merely in their clinical manifestations, but also in their morphology and on the level of molecular genetics yet significant to distinguish due to varying prognoses and treatment options.

Generally, and from a clinical point of view, lymphoid malignancies are either presented as leukemias or as lymphomas, depending on the primarily affected compartment. Leukemias primarily affect the bone marrow and peripheral blood, lymphomas primarily affect secondary lymphatic organs, e.g., lymph nodes. However, for the latest classifications, the division into leukemia or lymphoma is not important and the classification is based on precise phenotypic characterization of the malignant cells.[18] This supports the fact that, for example, chronic lymphocytic leukemia (CLL) tends to affect lymph nodes, and conversely, many lymphomas are associated with malignant clones present in bone marrow or peripheral blood. Clinically, the presence of lymphoma malignant cells in the peripheral blood is called leukemization, and it is quite common for some type of lymphomas, e.g., follicular lymphoma.[19,20]

Over 60 different types of lymphomas and lymphocytic leukemias have been described according to the latest World Health Organization (WHO) revision of the classification of lymphoid malignancies (from 2016).[18]

5 Non-Hodgkin lymphoma

NHL could be divided into more than 50 different types representing 80-90% of all lymphomas. NHL may be clinically divided by grade into high-grade (aggressive) lymphomas, including e.g., diffuse large B-cell lymphoma and Burkitt lymphoma, and low-grade (indolent) lymphomas, including marginal zone lymphoma, follicular lymphoma, and others.[18] NHL can originate from all types of lymphocytes (e.g., NK-Cells, B-Cells, and T-Cells); however, in more than 90% of all cases, they arise from B lymphocytes. The molecular classification of NHL is based on tumor

cell similarity to different stages of normal B cell development. Each subtype has a specific phenotype and changes in genetic information. (FIGURE 3)[18] The most frequent NHL types are described in the sections below.

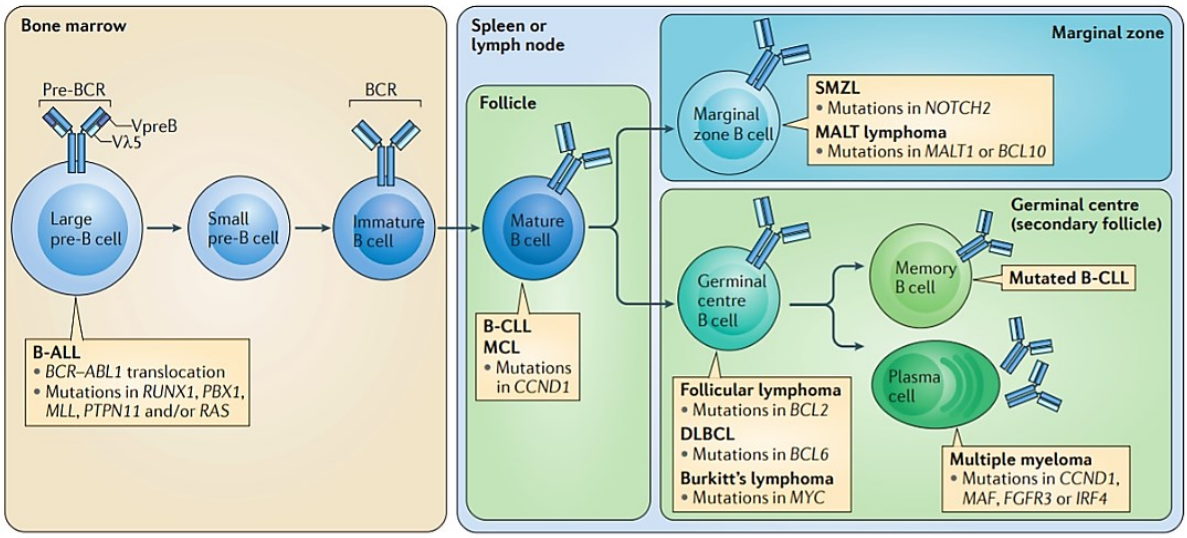

FIGURE 3. Development of B cell neoplasms at different stages of B cell differentiation. Source: Rickert et al. 2013,[96] with permission from Springer Nature.

5.1 Follicular lymphoma

Follicular lymphoma (FL) is the most frequent type of indolent NHL that originates from germinal center B cells and is characteristic for elderly patients with a median age of 65 years.[21] Germinal center is part of secondary lymphoid organs, where B cell differentiation and somatic hypermutation take place. Somatic hypermutation introduces mutations in the hyper-variable parts of Ig, which are needed for improving antigen affinity. Another process taking place in germinal centers is isotope switching, causing a change of the used type of the Ig constant region and thus the emergence of new types of Ig. The germinal center consists of light zone B cells called centrocytes (small lymphoid cells) and dark zone B cells called centroblasts (large lymphoid cells).[22] FL consists of both, centrocytes and centroblasts, and depending on their abundance is divided into grades: 1, 2, 3a, and 3b.[23]

Important for FL pathogenesis is t(14;18) translocation, during which the BCL-2 gene is translocated to the IgH region, leading to its overexpression. BCL-2 promotes B-cell survival by suppressing apoptosis.[23] However, other genetic alterations are necessary for malignant transformation as t(14;18) translocation is detectable also in healthy individuals.[24,14]

5.2 Diffuse large B-cell lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL, and as the name implies, it develops from B-cells.[21] Besides primary DLBCL, it could also develop by the transformation of the malignant cells of another lymphoma (e.g., FL).[25] DLBCLs are aggressive, heterogeneous lymphomas that differ in clinical manifestations and in the success of treatment, where chemotherapy proved to be effective only in 40% of all cases.[26]

This heterogeneity was partially explained by the gene expression profiling (GEP) studies, that revealed two main molecular subgroups according to the origin of the cell: the germinal center B-cell-like (GCB) and activated B-cell-like (ABC). Besides those two major subgroups of DLBCL, several cases remain "unclassified." In GCB DBLCL, the gene expression is similar to germinal center B cells, while ABC DBLCL expresses genes similar to activated and post-germinal center B cells. Generally, patients diagnosed with GCB DLBCL have better prognosis than those with ABC DBLCL.[26]

5.2.1 Classification of DLBCL according to gene characteristics

In certain types of DLCBL, some genetic aberrations occur more frequently than in others. According to these findings, four genetic subtypes of DLBCL have been described: MCD, BN2, N1, and EZB. Somatic mutations of CD79α and CD79β are characteristic for ABC DLBCL and are rare for other DLBCL subgroups. Of note, mutations of the CD79β are more common than mutations of CD79α. Another mutation that occurs mostly in ABC DLBCL is $MYD88^{L265P}$. Together these mutations represent the first genetic subtype called MCD. The BN2 subtype is characterized by BCL6 and NOTCH2 mutations that occur with the highest incidence in unclassified DLBCLs. BCL6 is one of the most frequent genetic aberrations in DLBCs, and it also appears with high frequency in other types of lymphoma. Both the MCD and BN2 subtypes are dependent on chronic active BCR signaling, which is described in more detail below. The N1 subtype is based on NOTCH1 mutations with 95% of them being ABC DLBCL. The last genetic subtype is named EZB and is characterized by EZH2 and BCL-2 mutations that mostly appear in GCB DLBCL. Distinguishing these genetic subgroups may be necessary for a better understanding of treatment.[27,28]

5.3 Mantle cell lymphoma

The mantle cell lymphoma (MCL) represents only 4-6% of all NHL.[21] It is an aggressive NHL developing from the mantle zone with a poor prognosis and it is considered as incurable. According to the newest WHO revision, MCL has two distinct molecular subtypes; classical

MCL and non-nodal MCL. Classical MCL, in contrast to leukemic non-nodal MCL, express SOX11 transcription factor and has no immunoglobulin heavy chain gene mutations.[18]

The critical mutation in MCL is translocation (11;14), resulting to the rearrangement of the BLC-1 gene, which leads to the overexpression of Cyclin-D, a significant cell cycle regulator.[29,30]

5.4 Burkitt lymphoma

Burkitt lymphoma (BL) is a highly malignant type of NHL originating from germinal center B-cells mostly affecting children. BL has a favorable prognosis with the chance of remission around 90% (in developed countries).[31] The characteristic change of genetic information in BL is MYC oncogene translocation. However, similar MYC deregulation is found in other lymphomas such as DLBCL.[32]

Denis Burkitt first described the disease in Africa, where the lymphoma occurred in children infected with Epstein Barr Virus (EBV) and malaria. There is a correlation between those diseases, where people infected with EBV have a much higher chance of developing BL. The type of BL which always associate with EBV and appears in malaria regions is called endemic. Another variant is sporadic, which mostly affects children, and in fewer cases, adults. The incidence of EBV in the sporadic variant is low. In addition, HIV-positive patients or, generally, people with immunodeficiency, for example, patients who take immunosuppressive drugs, have an increased risk of BL.[33,34,35]

6 Chronic lymphocytic leukemia

CLL is the most frequent type of leukemia in western countries. It is a disease with highly variable malignancy affecting B cells. In the recent classification, it is classified as a chronic lymphocytic leukemia / small lymphocytic lymphoma (CLL/SLL), highlighting its similarity to NHL.[18] The main feature of CLL is dysregulated cell proliferation, leading to an increased numbers of mature B cells and their subsequent accumulation in bone marrow, lymph nodes, blood, and spleen. It has been proven that monoclonal B-cell lymphocytosis precedes the vast majority of the CLL cases.[36] The most common genetic aberrations in CLL include deletions of 17p, 11q, 13q, and trisomy of chromosome 12.[37] A feature that divides CLL into two subgroups is whether or not it has mutated IGH V genes (referred to as mutated or non-mutated CLL). CLL thus develop either from naive B cells (unmutated HVR) or antigen-experienced memory B cells (mutated HVR). Mutated CLL is generally more indolent and has a better prognosis, while

unmutated CLL has a much more aggressive course of the disease with a need for early treatment.[38,39] An exception to this rule is a mutation in the VH3-21 gene, which shows an aggressive course of the disease similar to unmutated CLL.[40]

Of note is Richter syndrome, which is characterized by the transformation of low-malignancy CLL into aggressive NHL, mostly DLBCL.[41] CLL also includes small lymphocytic lymphomas (SLL), which differs from CLL in clinical manifestations. SLL is manifested primarily by an enlargement of the lymph nodes, but unlike CLL, it does not have an increased number of lymphocytes in the peripheral blood. However, the methods of treatment are the same for CLL and SLL.

7 Pathogenic BCR signaling in NHL

The viability of many NHLs is dependent on BCR signaling; therefore, this pathway is targeted by pharmaceutical inhibition. However, responses to the BCR-targeting drugs may differ for each type of lymphoma due to the variability BCR deregulation. Its dependence on the antigen can distinguish pathogenic BCR signaling into antigen-dependent signaling, including "chronic active" BCR signaling and My-T-BCR signaling and antigen-independent signaling, the so-called tonic signaling.

7.1 Chronic active BCR signaling

The first type of BCR signaling that promotes lymphoma survival is chronic active BCR signaling, discovered and described in ABC DLBCL. It is termed chronic active BCR signaling due to the similarity with BCR signaling in normal B cells after antigen binding.[42] Multiple signaling pathways are activated through chronic active signaling, such as PI3K and MAPK, however, the most important for ABC DLBCL survival is constitutive NF-κB activation and consequent activation of NF-κB dependent gene.[43] (FIGURE 6A) NF-κB is a family of transcription factors, which affects the expression of genes essential for cell development, apoptosis, immune system, and more. Constitutive activation of NF-κB is beneficial for lymphomas primarily by reducing apoptosis.[43] Normal B cells require protein CARD11 (also known as CARMA1) to activate NF-κB.[44] As a consequence of BCR activation, the CARD11 is phosphorylated by PKCβ[15], resulting in translocation to the sites of a plasma membrane. Near the plasma membrane, CARD11 associate with other proteins to form a CARD11-BCL10-MALT1 (CBM) complex.[45,46] Activated CBM complex recruits TNF receptor-associated factor (TRAF6), resulting in the activation of IκB kinase (IKK) and subsequent initiation of the NF-κB

pathway.[47] NF-κB is a protein dimer; its inactive form is located in the cytoplasm and is associated with IκBα, a family of proteins that inhibits the NF-κB activation. After the interaction of NF-κB with IKK, IκBα is marked with ubiquitin and subsequently degraded. The NF-κB dimers are then released from the cytoplasm to the nucleus, where they bind to their target genes and consequently affect the DNA transcription.

CARD11 mutations occur in ABC DLBCL in 10% of cases and are capable of inducing the NF-κB regardless of BCR signaling. Inhibition of BCR subunits (e.g., CD79α and CD79β) of cell lines with mutated CARD11 does not affect their ability to survive, supporting the idea of their independence from BCR signaling.[42] In contrast, activation of NF-κB in ABC DLBCL with wild-type CARD11 requires chronic active BCR signaling, and inhibition of BCR components is toxic to the lymphoma cells. Moreover, their BCRs form clusters in the plasma membrane resembling antigen-stimulated BCRs in normal B cells.[42]

A common mutation in ABC DLBCL (and also in MCL)[48] is the deletion of chromosome 6q[49] containing the TNFAIP3 gene, which directs the expression of NF-κB inhibitor, A20. The gene mutation or promoter methylation inactivate A20, therefore, promotes constitutive NF- κB signaling.[50] Of note, mutations in the TNFAIP3 gene were not found along with the mutated CARD11, suggesting that both genetic aberrations independently of each other perform the same function.[51]

Somatic mutations of CD79α and CD79β are characteristic for ABC DLBCL and are rare for other DLBCL subgroups. Prevalently mutated is CD79β (about 20% of cases), with a mutation affecting the ITAM regions by amino acid substitutions. In contrast, the mutations of CD79α (in about 3% of cases) cause the deletion of multiple amino acid residues.[42] These mutations support the expression of BCR on the cell surface and also suppress the BCR inhibitor (Lyn), resulting in promoting BCR activation with consequent higher level of NF-κB activity. The mutated CD79α and CD79β are able to increase the efficiency of BCR signaling, but they are not capable of initiating the signaling de novo.[42]

7.1.1 Binding of a self-antigen to BCR

As it was previously mentioned, chronic active BCR signaling is similar to antigen triggered BCR signaling in normal B-cells. In both cases, BCR signaling is antigen-dependent, activates same signaling cascades (e.g., PI3K, NF-κB), and their BCRs form similar clusters on the plasma membrane.[42] In normal B cells, activation of BCR is limited by the amount of available foreign antigens and by interactions with costimulatory and coinhibitory receptors. In contrast, ABC DLBCL requires constitutive BCR activation.[42] Therefore, the BCRs of ABC DLBCL cells bind

to antigens that originate from the tumor cells itself or are present in the tumor microenvironment and are not normally recognized by the immune system (self-antigens).[52] The binding of self-antigens can explain the formation of BCR clusters in ABC DLBCL.[42]

Binding of a self-antigen occurs not merely in ABC DLBCL, but all lymphoid malignancies utilizing chronic active BCR signaling for survival (e.g., CLL, MCL).[53,54,55] There are exceptions in which the lymphoma BCR recognizes and its growth is supported by foreign antigen. These cases are mostly associated with bacterial or viral diseases, such as hepatitis C virus in splenic marginal zone lymphoma[56] and *Helicobacter pylori* in marginal zone B-cell lymphoma.[57]

Antigen binding is mediated by antigen-binding sites of the IgH and IgL V domains. The V domain consists of three complementarity determining regions (CDRs) and four relatively constant sequences called framework regions (FRs). (FIGURE 4) In normal B and T cells, CDRs are highly variable regions that are needed for wide variability in antigen binding. FRs are less variable and are important primarily for V domain stabilization and promoting antigen binding to CDRs.[58] The type of IgH V domains plays a significant role in the binding of a self-antigen. Different V domains vary in the ability to bind various antigens due to the diversity of its structure, thereby affecting the viability of lymphoma.[52] In some lymphoma cells, specific IgH V genes are more common than others. ABC DLBCL most often expresses IgH V gene VH4-34,

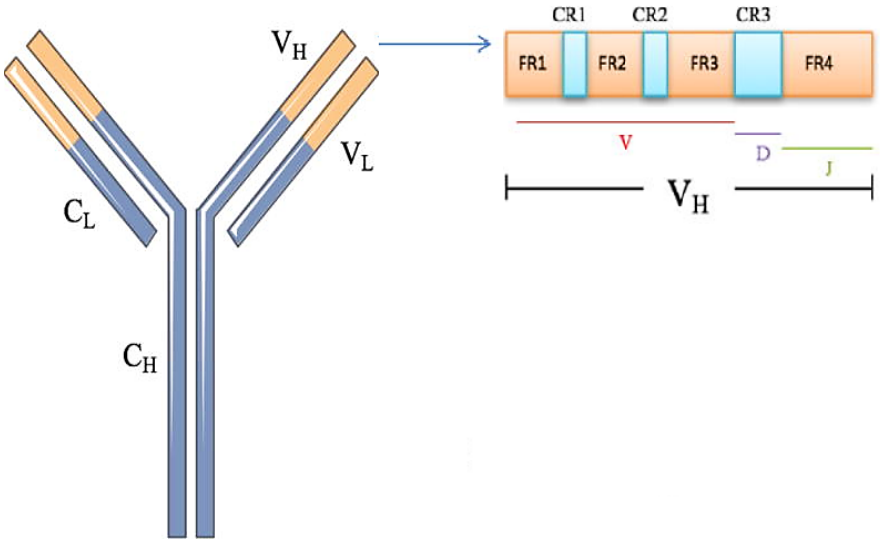

FIGURE 4. A model of an immunonglobulin, focusing on the struture of variable domain (V_H), which consists of three CDRs regions and four FRs. The model also shows the correlation between those regions and VDJ genes.
Source: Crombie et al. 2007,[97] with permission from John Wiley and Sons.

which is also rare in other types of DLBCLs and normal B-cells. Another more frequently used IgH V gene in ABC DLBCL is VH3-7, which, together with VH3-34, represents 39% of all cases in ABC DLBCL.[52]

Mature antigen inexperienced B-cells express on their surface IgM and IgD isotypes of BCR. Isotype switching is a mechanism in which the constant chain of Ig is changed in a process called class switch recombination of the constant region coding genes, resulting in the formation of other isotypes of the BCR: IgG, IgA, and IgE. Isotype switching takes place in the germinal center and involves B cells that have been activated by antigen binding to their BCR. Isotype switching depends on a switch μ region.[59] Mutations of the switch μ region occur in almost 50% of ABC DLBCL cases. Thus, in ABC DLBCL, although the lymphoma cells originate from post-germinal, activated B cells, IgM is expressed in 80% of cases. Moreover, ABC DLBCL cells that utilizing IgH VH3-34 express IgM on their surface in 90% of cases.[52] In contrast, mutations in the switch μ region are rare for GCB DLBCL, and, therefore, the most common BCR isotype in IgG.[60] Signaling of BCR with different isotypes differently affects the B-cell function. IgG primarily affects cell differentiation, therefore, retaining the IgM in ABC DLBCL may inhibit terminal B cell differentiation and promote lymphoma cell proliferation.[52] VH3-34 is the most frequently expressed IgH V gene also in other types of lymphoma, such as CLL[53,54] and MCL.[55]

Another example of self-antigen binding is in CLL. One-third of cases use stereotyped BCRs with similar IgH V segments,[54] and approximately 1% of cases express identical BCRs.[61] These data suggested that there is a correlation between the binding of specific antigens and the pathogenesis of CLL. Marcus Duhren-von Minden et al.[62] revealed that CLL utilizes autonomous antigen-independent signaling mediated by the IgH CDR3 segment and its interaction with an internal BCR epitope.

IgM+ cell lines derived from ABC DLBCL, HBL1, OCI-Ly10, and TMD8, are commonly used for research in lymphoma biology. The cell lines differ in their IgH V segments and their survival depends on both the BCRs on their surface and the IgHV segments appropriate to the individual lines.[52] This confirms the fact that DLBCL cells are dependent on chronic active BRC signaling and not on tonic signaling, where BCR expression with any IgH V would be sufficient. The HBL1 cell line expresses IgH VH3-34, which is the dominant IgH V gene among ABC DLBCL. It binds glycoproteins on its cell surface as self-antigens. (FIGURE 5) The self-glycoproteins are recognized via the FR1 and CDR3 segments.[52] OCI-Ly10 expresses IgH VH7-3, the second most common IgH V in ABC DLBCL, and binds self-antigens released from apoptotic debris. The last cell line, TMD8, expresses the IgH V gene VH3-48, which was not shown to be dominant in any subgroups of lymphoma. The BCR of TMD8 binds to an idiotope

via the FR2 segment.[52] (FIGURE 5) An idiotope is an epitope (antigenic determinant) on the Ig V domain and is unique for a particular Ig. A set of idiotopes of one Ig chain form an idiotype.

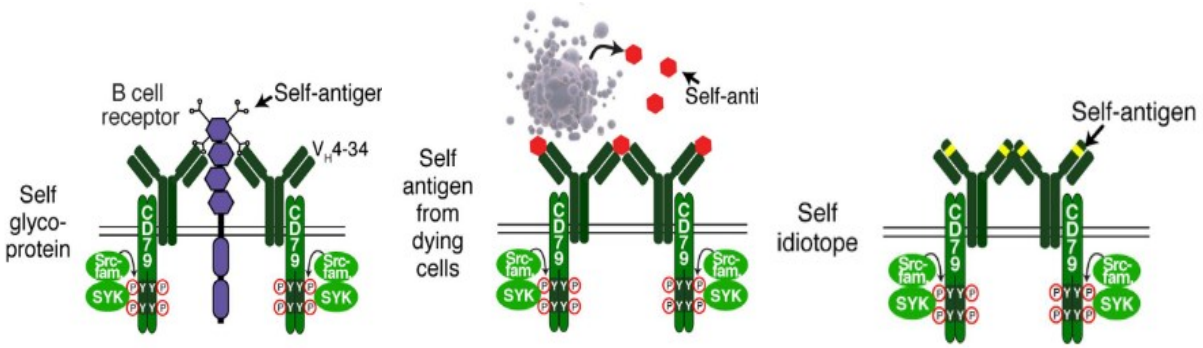

FIGURE 5. Types of self-antigens used by different cell lines derived from ABC DLBCL; HBL1 (VH3-34) bind self-glycoproteins to it BCR, OCI-Ly10 (VH7-3) recognize self-antigens realesed from dying cells and TMD8 (VH3-48) whose BCR binds to an idiotope.
Source: Young et al. 2019,[98] with permission from John Wiley and Sons.

7.2 My-T-BCR complex

Mutated CARD11 and BCR signaling are two ways lymphoma cells can activate the NF-κB pathway.[42] The third way to activate NF-κB is also dependent on BCR signaling and, in addition, on protein MYD88.[63] In normal cells, MYD88 is involved in interleukin-1 (IL-1) receptor and tool-like receptor (TLR) signal transduction. Stimulation of TLR and IL-1 receptors leads to the clustering of a family of interleukin-1-associated kinases (IRAK) near MYD88. The first protein associating with MYD88 is IRAK4, followed by activation of IRAK1.[64] Engaged IRAK1 recruits TRAF6 and TAK1, resulting in the stimulation of two signaling cascades NF-κB and MAPK.[65,66]

Mutated MYD88 isoforms occur in ABC DLBCL in 39% of cases. These mutations are mostly affecting the Toll / IL-1 receptor (TIR) domain at position 265, where proline is substituted for leucine (referred to as MYD88 P265L).[63] The primary oncogenic function of MYD88 P265L in ABC DLBCL is the activation of the NF-κB pathway. Moreover, the intensity of the activation by MYD88 P265L is much higher than by wild-type MYD88.[63] In most cases, the mutation of CD79β occurs along with the mutation of MYD88 P265L. The co-occurrence of these mutations is more common than mere coincidence, suggesting a link between MYD88 P265L and BCR.[63]

A significant receptor interacting with MYD88 is TLR9, which recognizes CpG sites, mostly found in bacterial or viral DNA.[67,68] In normal B-cells, upon activation of TLR9, the receptor is relocated to the endoplasmatic reticulum. From there, the receptor is transferred to lysosomes, where it forms a functional complex with MYD88, essential for subsequent TLR9 signaling.[69] In ABC DLBCL, the TLR9 receptor is operably linked to a complex consisting of MYD88 and BCR, the so-called MY-T-BCR complex.[70] (FIGURE 6B) The assembly of the complex takes place on the endolysosome membrane, where the BCR is transferred from the cell surface by internalization. The primary function of the MY-T-BCR complex is to initiate most of the NF-κB pathway in B-cell.[70] Moreover, the CBM complex co-associate with the MY-T-BCR complex and contributes to the regulation of the NF-κB pathway.[71]

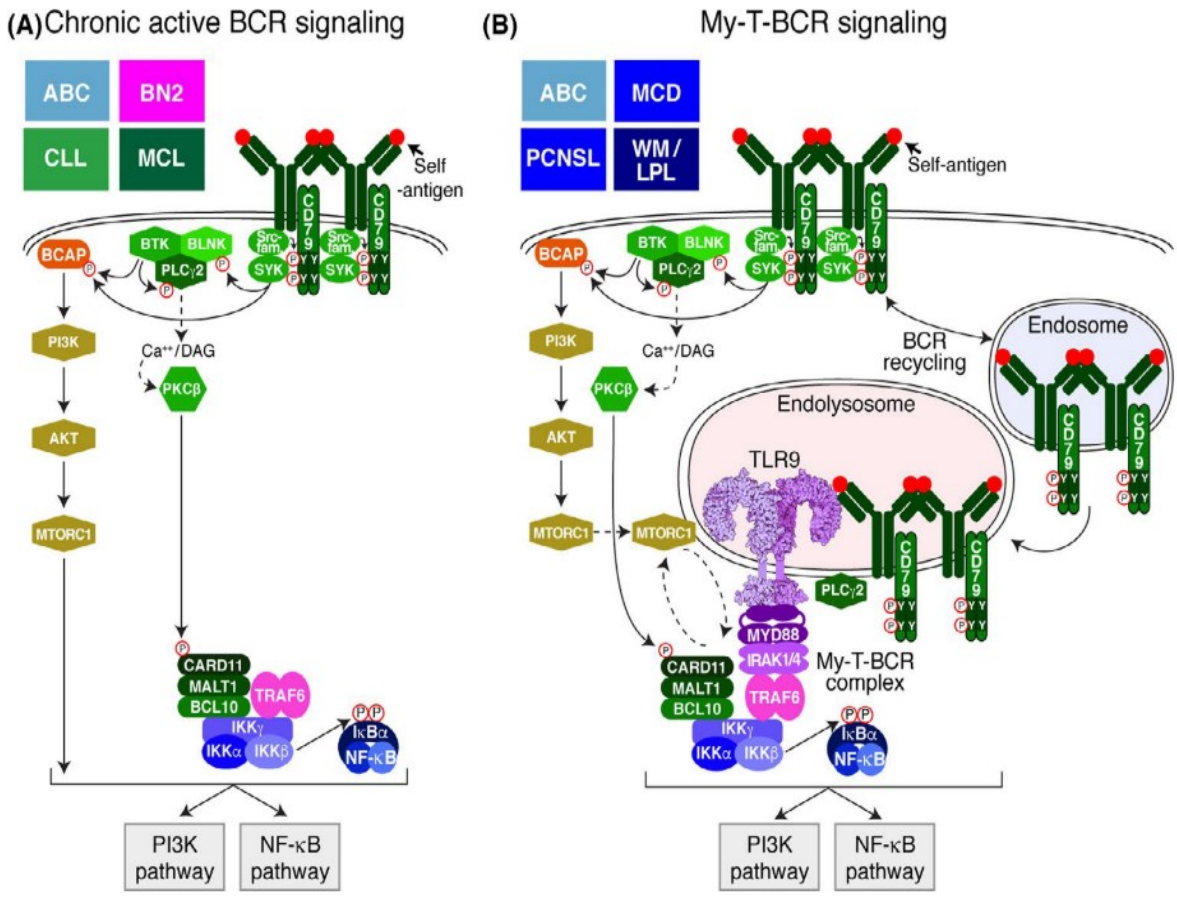

FIGURE 6. Two types of pathogenic BCR signaling in the NHL. Both types are dependent on chronic active BCR signaling, which is mediated by self-antigen binding to their BCR.
Source: Young et al. 2019,[98] with permission from John Wiley and Sons.

7.3 Tonic BCR signaling in NHL

As mentioned above, the BCR transmit low-level tonic signals not dependent on prior antigen binding, which is about four times weaker than antigen-stimulated BCR signaling, yet are essential for the survival of all mature B cells.[17,72]

In normal B cells, a tonic signal is transmitted through CD79α and CD79β molecules, phosphorylation of SYK kinase and CD19 mediated activation of the PI3K/AKT pathway. (FIGURE 7) AKT then directly phosphorylates FoxO1 transcription factor, leading to its inactivation and cell cycle and apoptosis modulation. FoxO1 is a member of the forkhead box O (FoxO) family of transcription factors that are essential especially for regulation of various critical cellular processes, including the regulation of apoptosis, metabolism, and the cell cycle. Unlike antigen-driven BCR signaling, tonic signaling does not activate the NF-κB pathway and PI3K/AKT activation together with FoxO1 is sufficient for the survival of resting B cells.[2,17]

The viability of certain NHLs depends on the presence of tonic BCR signaling. Such NHLs are GCB DLBCL and BL. Although both lymphomas originate from germinal center B cells, they differ in the pathogenesis of tonic BCR signaling. In addition to PI3K signaling, they also require CD79α and SYK for its activation and cell survival.[73,74] Inhibitors of the PI3K pathway are also very effective in the treatment of BL. The most common mutations supporting constitutive tonic BCR signaling in BL affect TCF3 transcription factor and its inhibitor ID3. TCF3 suppresses the expression of PTPN6 tyrosine phosphatase, a significant inhibitor of BCR signaling.[73]

A small part of GCB DLBCL has been shown to have BCRs capable of initiating signaling similar to antigen-dependent signaling. However, most GCB DLBCLs, unlike ABC DLBCL, do not form BCR clusters on their cell surface, mutations in CD79α and CD79β molecules that promote antigen-dependent BCR signaling are rare, and they lack NF-κB dependency. All of these features are consistent with the fact that the GCB subtype uses antigen-independent BCR signaling.[42] GCB subtype depends on active AKT, which is primarily regulated by tonic BCR signaling requiring phosphorylation of CD79α Y188. Moreover, approximately 50 % of CGB DLBCL lack PTEN, a negative regulator of the PI3K/AKT pathway, leading to its signal enhancement.[13,75] In contrast to the uniform dependency of GCB DBCL cells on AKT activity, not all CDB DLBCL tumors are dependent on BCR. The degree of PI3K/AKT activation from BCR is directly correlated with the BCR surface density and the PTEN status. The PTEN negative and/or low BCR cells tend to be independent on PI3K/AKT activation from BCR. This may be the reason for uneven efficacy of SYK inhibitors in GCB DLBCL and need for precise biomarkers for personalized treatment.[75]

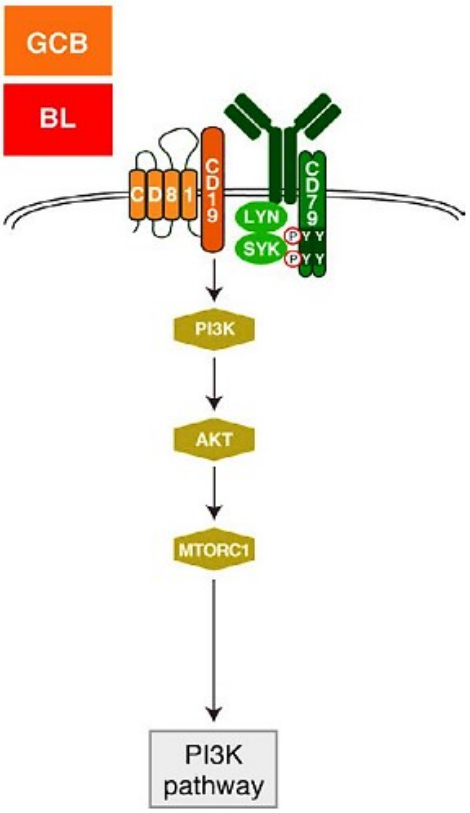

FIGURE 7. Tonic BCR signaling transmitted via CD79α and CD79β molecules, phosphorylation of SYK kinase and CD19 mediated activation of the PI3K/AKT pathway.
Source: Young et al. 2019,[98] with permission from John Wiley and Sons.

8 BCR inhibitors as a therapy in lymphoid malignancies

Due to the important role of BCR in the survival of many lymphoid malignancies, inhibition of BCR signaling members (most commonly targeted to kinases) appears to be the right direction for their future therapy. Many BCR inhibitors are in clinical trials, and some are already in clinical practice. The effectiveness of treatment by individual inhibitors varies in distinct types of lymphoma, depending on the type of BCR signaling.

8.1 BTK

Inhibitors of BTKs are effective in lymphomas that are dependent on active BCR signaling, e.g., in lymphomas using chronic active BCR signaling. In contrast, for GCB DLBCL, which uses predominantly tonic BCR signaling, this type of inhibitor is ineffective.[42] The first clinically used BTK inhibitor is ibrutinib, which binds covalently to cysteine 481 near the active site of BTK. However, it does not bind very specifically as many off-targets were described.[76] Ibrutinib

was approved by the U.S. Food and Drug Administration (FDA) in the treatment of MCL (in 2013), CLL (in 2014).[77]

Clinical trials have shown that ibrutinib is effective in 37% of ABC DLBCL cases, compare to only 5% in GCB DLBCL. The most significant results with up to 80% efficiency were found in ABC DLBCL harboring mutations in CD79β and MYD88 (most commonly MYD88 P265L).[78] Another approved BTK inhibitor is acalabrutinib, which binds more specifically to BTK and has fewer side effects than ibrutinib. Based on phase 2 and 3 clinical trials, acalabrutinib was approved for the use in the treatment of MCL (in 2017)[79] and CLL (in 2019).[80,81]

8.2 SYK

SYK mediates signaling in both types of BCR signaling, in the chronic active BCR signaling as well as in the tonic BCR signaling. This makes it a promising target for NHL treatment.[42,74] Several SYK inhibitors are currently in clinical trials (or have been discontinued), but none has yet been approved for the treatment of lymphoid malignancies. The most studied SYK inhibitor is fostamatinib, which turned out to be most effective in CLL but also had some activity in DLBCL, MCL, and FL.[82] In addition, fostamatinib is the first approved SYK inhibitor, prescribed to patients with chronic immune thrombocytopenia.[83] Of note is an SYK inhibitor entospletinib, with higher selectivity than fostamatinib, currently in clinical trials for the treatment of CLL, DLBCL, MCL, and others.[84,85,86]

8.3 PI3K

PI3K/AKT signaling is also induced in both types of BCR signaling utilized by NHLs. Unlike SYK inhibitors, there are more PI3K inhibitors approved for the use in the treatment of lymphoid malignancies, targeting mostly PI3K class I. The inhibitors differ in their selectivity for individual catalytic subunits of PI3K (p110α, p110β, p110γ, and p110δ), but mostly targeting the PI3Kδ (the B-lymphocytes predominant isoform). The first approved inhibitor is idelalisib (in 2014), which inhibits PI3Kδ and is used in the treatment of CLL and FL.[87] However, with quite high toxicity.[88] Another example of an approved inhibitor is copanlisib, which targets PI3Kα and PI3Kδ, and is used in the treatment of relapsed FL.[89,90] The latest approved PI3K inhibitor is duvelisib (in 2018), selective for PI3Kδ and PI3Kγ, used in the treatment of CLL and FL.[91] A number of inhibitors are currently in clinical trials, such as umbralisib. It is highly selective not only to PI3Kδ, but also to casein kinase 1 epsilon, which may lead to a higher toxicity.[92]

BCR signaling inhibitors are often combined with other inhibitors or drugs for better treatment outcomes. Examples are idelalisib and duvelisib, which are used in combination with rituximab, an anti-CD20 monoclonal antibody, for better efficacy in the treatment of CLL.[93,91]

9 Conclusion

Dysregulation of BCR signaling plays a key role in the tumorigenesis and survival of many B-cell derived malignancies (mainly NHLs and CLL). A common model for research of BCR signaling is the most frequent type of NHL, the DLBCL. GEP studies have revealed its two subtypes differing at the molecular level: ABC DLBCL, whose gene expression resembles activated B cells, and GCB DLBCL, resembling the germinal center B-cells. Although these subtypes are classified as one disease, they differ fundamentally in their response to the treatment.[26] Moreover, studies have classified DLBCL also according to the occurrence of the most frequent mutations into 4 subgroups, MCD, BN2, N1, and EZB, with an interest in developing more effective and personalized treatment.[27,28]

Multiple signaling pathways are activated by BCR signaling in lymphomas. The chronic active BCR signaling activates PI3K, MAPK, and most importantly NF-κB. Certain NHLs have genetic mutations promoting the constitutive BCR signaling e.g., mutated CD79α/β BCR co-receptor molecules in ABC DLBCL. The initiation of chronic active BCR signaling is mostly mediated by the binding of self-antigens, which is driven by the structure of IgG V genes. Of note is the CARD11 mutation occurring in 10% of ABC DLBCL cases, which has the ability to initiate the NF-κB pathway and thus constitutive BCR signaling without prior antigen-binding.[42,52]

Another way of chronic active BCR signaling initiation in ABC DLBCL is through MYD88. MYD88 forms a functional complex with BCR and TLR9 on the endolysosomal membrane with consequent induction of most of the NF-κB pathway. Of note is the co-occurrence of the MYD88 and CD79β mutations in ABC DLBCL.[63]

The BL and GCB DLBCL tumor cells utilize tonic type of BCR signaling with high dependence on its downstream PI3K/AKT pathway. In BL, this pathway is promoted by mutations in TCF3 and ID3, and treatment with PI3K inhibitors is very effective.[73] In GCB DLBCL, the regulation of the PI3K/AKT pathway is more complex and depends on BCR density and PTEN status. GCBs that lack PTEN or have low-density BCR tend to be independent of BCR mediated activation of PI3K/AKT signaling. This corresponds to the variable response to upstream BCR signaling inhibition by SYK inhibitors.[75]

Due to their high specificity, BCR inhibitors are very promising treatment modalities for B-lymphoid malignancies. Clinically are used mainly BTK inhibitors in the treatment of MCL and CLL (ibrutinib and acalabrutinib)[77,80,81,79] and PI3K inhibitors in the treatment of CLL and

FL (idelalisib and duvelisib).[87,91] Many BCR related inhibitors are currently in clinical trials, including promising SYK inhibitor entosplentib.[84,85,86] The efficacy of treatment by individual inhibitors varies in distinct types of lymphoma, depending on the type of BCR signaling.

Understanding of the molecular nature of different types of B-lymphoid malignancies is critical for specific and more personalized treatment. This is highlighted by the importance and complexity of BCR signaling and related complexity of its dysregulation. A further and even deeper understanding of tumor-specific signaling and processes should, hopefully, mediate further therapy improvements.

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